Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 1291–1295
Bioorganic & Medicinal Chemistry Letters

3-Substituted-(5-arylfuran-2-ylcarbonyl)guanidines as NHE-1 inhibitors

Sunkyung Lee,[a] Taemi Kim,[a] Byung Ho Lee,[a] Sung-eun Yoo,[a] Kyunghee Lee[b] and Kyu Yang Yi[a,*]

[a]*Bio-organic Science Division, Korea Research Institute of Chemical Technology, Yuseong-gu, Daejeon 305-600, Republic of Korea*
[b]*Central Research Center of Yuyu Inc., Anyang-si, Gyeonggi-do 430-804, Republic of Korea*

Received 13 July 2006; revised 23 November 2006; accepted 4 December 2006
Available online 15 December 2006

Abstract—The C-3 substituents effect on NHE-1 inhibitory activity of (5-arylfuran-2-ylcarbonyl)guanidines, previously identified as potent NHE-1 inhibitors, was investigated. The introduction of amine or alkyl groups at the 3-position of the furan ring, next to the acylguanidine moiety, remarkably improves NHE-1 inhibitory potency. Especially the important finding is that 5-(2,5-dichloro)phenyl and 5-(2-methoxy-5-chloro)phenyl derivatives exhibit high NHE-1 inhibitory activities ($IC_{50} < 0.02$ μM) that match those of 3-unsubstituted derivatives.

The Na^+/H^+ exchanger isoform-1 (NHE-1) is known to regulate intracellular pH (pHi) during ischemia. In spite of its essential role in restoring normal pHi,[1] activation of NHE-1 has been implicated in deleterious consequences that occur during ischemia and reperfusion, such as contractile dysfunction, arrhythmia, and cell death. Consequently, inhibition of the NHE-1 has been identified as a strategy for reducing ischemia/reperfusion injury.[2]

In an earlier study, we found that members of a series of (5-arylfuran-2-ylcarbonyl)guanidines exhibit good inhibitory activity toward NHE-1 and both in vitro and in vivo cardioprotective efficacy against ischemia/reperfusion injury.[3] The effect of substitution at the 5-aryl position was examined in that effort. Earlier reports suggest that functionality adjacent to the acylguanidine moiety leads to improved NHE-1 inhibitory potency, presumably due to an increase in hydrophobic interactions and/or reduction in the conformational flexibility of the acylguanidine group.[4–6] Herein, we summarize the results of studies evaluating NHE-1 inhibition by (5-arylfuran-2-ylcarbonyl)guanidines that contain amine, alkyl and aryl groups at the 3-position of the furan ring.

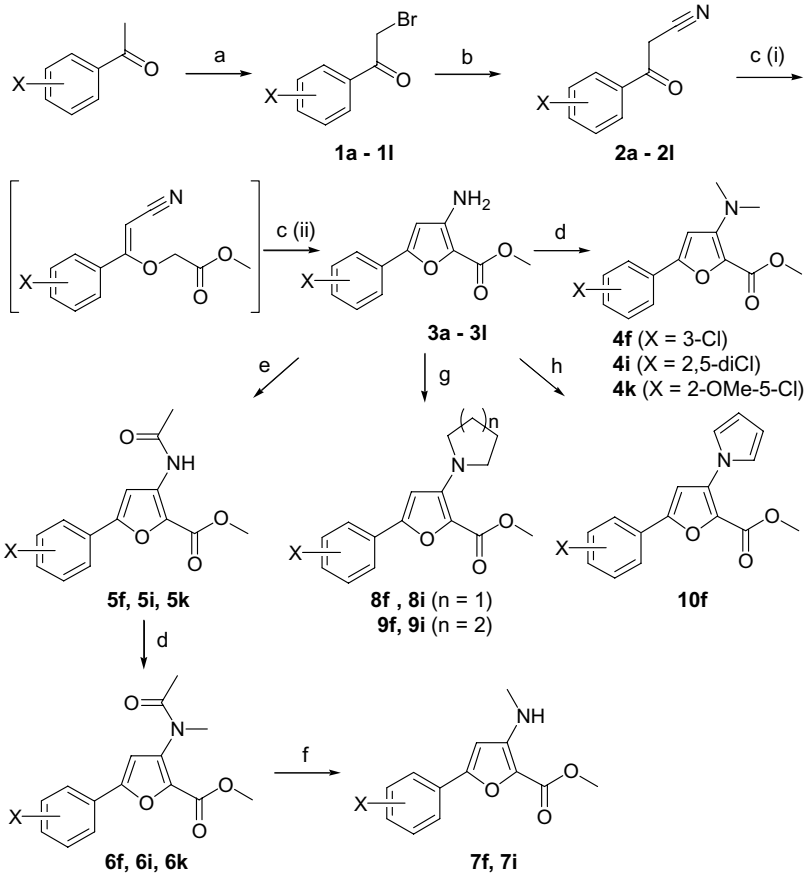
5-Aryl-3-aminofuran-2-carboxylate esters **3** (Scheme 1), key intermediates in pathways for synthesis of the amine substituted furan-2-carbonylguanidines, were prepared from the corresponding acetophenones via a sequence initiated by α-bromination[7] and cyanide displacement to form the 2-cyanoacetophenones **2**.[8] Reaction of **2** with methyl glycolate under Mitsunobu conditions afforded the vinyl ethers which upon treatment with sodium hydride cyclized to the 3-aminofurans **3** (40–60%).[9] The amine function in **3** was modified by using conventional methods to produce *N*,*N*-dimethylamine **4**, *N*-acetamide **5**, *N*-methylacetamide **6**, *N*-methylamine **7**, pyrrolidine **8**, piperidine **9**, and pyrrole **10**.

The intermediate 3-alkyl or 3-arylfuran-2-carboxylate esters **14–16** were prepared by Darzen condensation of the corresponding β-keto acetals with methyl chloroacetate followed by thermolytic conversion of the resulting glycidic esters **11–13** (Scheme 2).[10] The furan rings in **14–16** were then brominated at C-5 setting the stage for synthesis of 5-aryl derivatives **20–22** by Pd-catalyzed Suzuki coupling.[11]

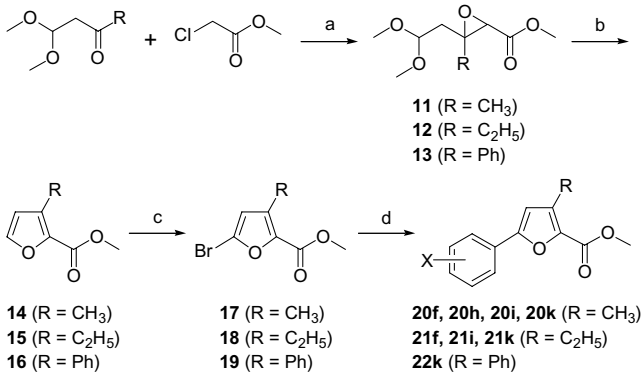
The 3-substituted (5-arylfuran-2-ylcarbonyl)guanidines **23–33** were then prepared from the corresponding carboxylate esters by the treatment with excess guanidine

Keywords: Sodium hydrogen exchanger; 3-Substituted-(5-arylfuran-2-ylcarbonyl)guanidine; Cardioprotective.
* Corresponding author. Tel.: +82 42 860 7143; fax: +82 42 861 1291; e-mail: kyyi@krict.re.kr

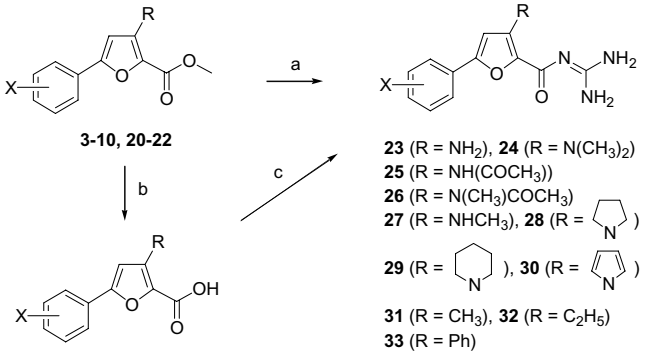
0960-894X/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2006.12.012



Scheme 1. Reagents and conditions: (a) Br_2, Et_2O, rt, 70–90%; (b) NaCN, EtOH, H_2O, rt, 80–90%; (c) i—Ph_3P, DEAD, methyl glycolate, THF, 0 °C → rt; ii—NaH, rt, 40–60%; (d) CH_3I, NaH, DMF, 0 °C → rt, 50–70%; (e) Ac_2O, 90–95%; (f) 6 N HCl, reflux, 45–60%; (g) $Br(CH_2)_nBr$ (n = 5, 6), NaH, DMF, 0 °C → rt; 45–65%; (h) 2,5-dimethoxytetrahydrofuran, 4-chloropyridinium hydrochloride, dioxane, reflux, 60–80%.



Scheme 2. Reagents and conditions: (a) $NaOCH_3$, THF, −10 °C to rt, 80%; (b) heating, 160 °C, 85%; (c) Br_2 Et_2O, rt, 78%; (d) arylboronic acid, $Pd(Ph_3)_4$, $Ba(OH)_2{\cdot}H_2O$ or K_2CO_3, toluene or DME, reflux, 60–75%.

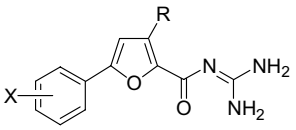


Scheme 3. Reagents and conditions: (a) guanidine, DMF, rt, 50–80%; (b) 1 N NaOH, CH_3OH, reflux, 85–95%; (c) i—CDI, THF, rt; ii—guanidine, rt, 55–70%.

in DMF (Scheme 3). Alternatively, these substances can be produced by activation of the respective carboxylic acids with 1,1′-carbonyldiimidazole (CDI) followed by treatment with guanidine.

The NHE-1 inhibitory activities of the synthesized compounds were determined by measuring their effect on the sodium-dependent recovery of pH following an imposed acidosis in PS120 variant cells in which the human NHE-1 is selectively expressed.[12] By using this method, the IC_{50} value of cariporide was 1.2 μM (Table 1). The IC_{50} values of 3-amino-5-phenylfurans **23** were compared with those determined earlier for the corresponding 3-unsubstituted derivatives.[3] The results show generally that introduction of a 3-amino group remarkably enhances NHE-1 inhibitory potency. This is especially true for the 2,5-dichloro **23i** and 2-methoxy-5-choro **23k** derivatives which have excellent inhibitory

Table 1. NHE-1 inhibition by 3-amino (**23**) and 3-methyl (**31**) furanylcarbonyl-guanidines



X	R = NH_2		R = CH_3		R = H
	Compound	IC_{50}[a] (μM)	Compound	IC_{50}[a] (μM)	IC_{50}[a,b] (μM)
Cariporide		1.2			
H	**23a**	2.3			3.1
2-F	**23b**	1.2			3.5
2-CH_3	**23c**	0.24			0.34
2-OMe	**23d**	0.15			2.3
3-F	**23e**	0.68			2.4
3-Cl	**23f**	0.15	**31f**	0.024	0.75
3-CH_3	**23g**	0.77			6.0
2,5-DiF	**23h**	0.41	**31h**	0.13	1.3
2-5-DiCl	**23i**	0.008	**31i**	0.016	0.12
2,5-DiCH_3	**23j**	0.072			0.65
2-OMe-5-Cl	**23k**	0.015	**31k**	0.010	0.081
2-OMe-5-F	**23l**	0.12			0.48

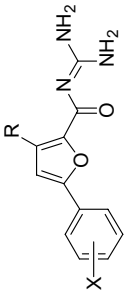
[a] Values are means of three experiments.
[b] Values are from previous report.

properties (IC_{50} < 0.02 μM). Moreover, the effects of C-5 aryl ring substituents in the 3-aminofuran series on IC_{50} values parallel those of the 3-unsubstituted compounds. Additionally, the 3-methylfurans **31** show similarly increased inhibitory activities. These results are in accord with the earlier suggestion that introduction of an additional substituent adjacent to the acylguanidine would lead to increased NHE-1 inhibition.[4]

The influence of various 3-substituents on the NHE-1 inhibitory activity was determined (Table 2). The 3-*N*,*N*-dimethylamino compounds **24** are as potent inhibitors as the corresponding primary amines, while 3-*N*-monomethylamine analogs **27** have slightly lower potencies. Inhibition by the 3-acetamides **25** and **26** is dramatically lower than that of the related amines, an observation which differs from that described in a published report.[13] The 3-pyrrolidine **28**, piperidine **29**, and pyrrole **30** analogs do not have improved activities. In addition, the 3-phenyl analog **33k** (IC_{50} = 5.6 μM) was not as active as its 3-unsubstituted counterpart (IC_{50} = 0.081 μM). Although the 3-ethyl derivative **32f** (IC_{50} = 0.23 μM) is three-times more potent than the unsubstituted analog (IC_{50} = 0.75 μM), it is not as active as the 3-methyl compound **31f** (IC_{50} = 0.024 μM). The combined results demonstrate that the 3-amino, 3-*N*, *N*-dimethylamino, 3-methyl compounds have greatly increased NHE-1 inhibitory activities and similar responses to C-5 phenyl ring substitution as compared to 3-unsubstituted derivatives.

The in vitro and in vivo cardioprotective efficacies against ischemia/reperfusion injury of the furan-2-carbonylguanidines that have good NHE-1 inhibitory activities were evaluated (Table 3). The isolated rat heart model was used.[14] Each isolated rat heart was treated with 10 μM of the compound for 10 min, subjected to 30 min global ischemia followed by 30 min reperfusion. The evaluation of the cardioprotective effect was measured as an index of cardiac contractile function based on the percent recovery of rate pressure product (RPP, HR × LVDP, heart rate × left ventricular developing pressure) at the end of reperfusion to the pre-ischemic value. Additionally left ventricular end diastolic pressure (LVEDP) was used as an indicator of cardiac contracture. In this model, cariporide significantly improves the recovery of contractile function (48% RPP) and diminishes LVEDP (24 mmHg) compared with the vehicle group (13% RPP, 56 mmHg LVEDP). Cardioprotective in vivo efficacy was determined by measuring a ratio of myocardial infarction size to area at risk (IS/AAR) by using a rat myocardial infarction model[15] that was stabilized for 20 min after a left thoracotomy operation, subjected to 45 min coronary artery occlusion, following 90 min reperfusion. The compounds (0.1 mg/kg) were administered by bolus intravenous injection 5 min before onset of ischemia. In this model, cariporide has a 41% of IS/AAR corresponding to a significant reduction of the infarct size compared with the vehicle group (59% of IS/AAR). Among the 3-amino derivatives, 2-fluorophenyl **23b** (62% RPP, 20 mmHg LVEDP) and 3-fluorophenyl **23e** (49% RPP, 25 mmHg LVEDP) showed a significant effect on recovery of cardiac contractility, that is similar to or slightly better than that of cariporide (48% RPP, 24 mmHg LVEDP). In a manner strikingly different from the 3-unsubstituted substances, most of the 3-amino analogs did not show any significant protective activity against ischemia–reperfusion injury in the Langendorff model. Especially, the most potent derivatives against NHE-1, 2-methoxy-5-chloro compounds (**23k**, **24k**, **31k**), were not active in the isolated rat heart model. The 3-unsubstituted analogs represented good correlation between NHE-1 inhibitory activity and cardioprotective efficacy,[3a] but not the 3-unsubstitued

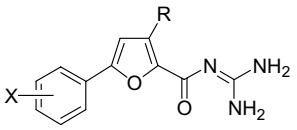
Table 2. NHE-1 inhibition by furanylcarbonyl-guanidines **23**-**33**



X	R = H	NH_2		$N(CH_3)_2$		NHAc		$N(CH_3)Ac$	$NHCH_3$							CH_3			C_2H_5		Ph	
	Compound, IC^{a}_{50} (μM)																					
H	3.1	**23a**	0.15	**24a**	0.12	**25a**	14	**26a**	>30	**27a**	0.82							**31a**	0.024			
3-Cl	0.75	**23f**	0.15	**24f**	0.12	**25f**	14	**26f**	>30	**27f**	0.82	**28f**	5.1	**29f**	16	**30f**	3.0	**31f**	0.024	**32f**	0.23	
2,5-DiCl	0.12	**23i**	0.008	**24i**	0.009	**25i**	6.2	**26i**	>30	**27i**	0.096	**28i**	0.48	**29i**	0.48			**31i**	0.016			
2-OMe-5-Cl	0.081	**23k**	0.015	**24k**	0.012	**25k**	4.5	**26k**	33									**31k**	0.010			**33k**	5.6

[a] Values are means of three experiments. The IC_{50} value of cariporide was 1.2 μM.

Table 3. Cardioprotective efficacy against ischemia–reperfusion injury



Compound	R	X	Langendorff[a]		In vivo[b]
			RPP (%)	LVEDP (mmHg)	IS/AAR (%)
Control			13 ± 1.3	56 ± 2.2	59 ± 1.5
Cariporide			48 ± 5.1	24 ± 3.2	41 ± 2.1
23b	NH_2	2-F	62 ± 10.7	20 ± 5.2	
23c	NH_2	2-CH_3	30 ± 6.3	28 ± 4.2	47 ± 1.8
23d	NH_2	2-OMe	30 ± 9.7	31 ± 8.8	55 ± 1.0
23e	NH_2	3-F	49 ± 8.1	25 ± 4.7	
23f	NH_2	3-Cl	33 ± 3.4	27 ± 7.8	53 ± 0.9
23k	NH_2	2-OMe-5-Cl	27 ± 4.3	29 ± 2.9	
24k	$N(CH_3)_2$	2-OMe-5-Cl	6.4 ± 3.3	40 ± 6.1	
31k	CH_3	2-OMe-5-Cl	0.3 ± 0.3	34 ± 6.0	

[a] In vitro cardioprotective effect was evaluated by measuring % RPP (LVDP × HR) to pre-ischemic value, LVEDP (10 μM). $n = 3$ or higher.

[b] In vivo cardioprotective effect was determined by measuring a ratio of myocardial infarct size to area at risk (IS/AAR) in rat myocardial infarction model (0.1 mg/kg). Values are means, $n = 3$ or higher.

derivatives. Even none of compounds display protective efficacies in the rat myocardial infarction model. The discrepancy between NHE-1 inhibition and cardioprotective efficacy associated with these substances is not clear. However, since these properties are well-correlated in 3-unsubstituted analogs,[3a] the protective efficacy may be due to the inhibitory activity on NHE-1. There may be a possibility that the differences are presumably attributable to the 3-substituents, but it still needs more investigation.

In summary, a series of 3-substituted (5-arylfuran-2-ylcarbonyl)guanidines were synthesized and evaluated for their NHE-1 inhibitory activities and in vitro and in vivo cardioprotective efficacies. The NHE-1 inhibitory potencies of the 3-substituted analogs, especially 3-amino, 3-*N*,*N*-dimethylamino, and 3-methyl compounds, are much stronger than those of the 3-unsubstituted analogs. However, both the in vitro and in vivo cardioprotective efficacies against ischemia/reperfusion injury of these substances were not as good as that of the 3-unsubstituted analogs. Continuing studies are underway to identify the source of the discrepancy and to uncover new compounds with better cardioprotective profiles.

Acknowledgments

This research was supported by grants from the Center for Biological Modulators of the 21st Century Frontier R&D program, the Ministry of Science and Technology (CBM-01-A100-001-1-0-0) and the Ministry of Health & Welfare, Korea.

References and notes

1. Karmazyn, M.; Gan, X. T.; Humphreys, R. A.; Yoshida, H.; Kusumoto, K. *Circ. Res.* **1999**, *85*, 777.
2. Spitznagel, H.; Chung, O.; Xia, Q.-G.; Rossius, B.; Illner, S.; Jähnichen, G.; Sandmann, S.; Reinecke, A.; Daemen, H. J. A. P.; Unger, T. *Cardiovasc. Res.* **2000**, *46*, 102.
3. (a) Lee, S.; Yi, K. Y.; Hwang, S. K.; Lee, B. H.; Yoo, S.-e.; Lee, K. *J. Med. Chem.* **2005**, *48*, 2882; (b) Lee, B. H.; Seo, H. W.; Yi, K. Y.; Lee, S.; Lee, S.; Yoo, S.-e. *Eur. J. Pharmacol.* **2005**, *511*, 175; (c) Lee, B. H.; Yi, K. Y.; Lee, S.; Lee, S.; Yoo, S.-e. *Eur. J. Pharmacol.* **2005**, *523*, 101; (d) Kim, M. J.; Moon, C.-H.; Kim, M.-Y.; Lee, S.; Yi, K. Y.; Yoo, S.-e.; Lee, S. H.; Baik, E. J.; Jung, Y.-S. *Eur. J. Pharmacol.* **2005**, *525*, 1.
4. Baumgarth, M.; Beier, N.; Gericke, R. *J. Med. Chem.* **1997**, *40*, 2017.
5. Guzman-Perez, A.; Wester, R. T.; Allen, M. C.; Brown, J. A.; Buchholz, A. R.; Cook, E. R.; Day, W. W.; Hamanaka, E. S.; Kennedy, S. P.; Knight, D. R.; Kowalczyk, P. J.; Marala, R. B.; Mularski, C. J.; Novomisle, W. A.; Ruggeri, R. B.; Tracey, W. R.; Hill, R. J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 803.
6. Ahmad, S.; Doweyko, L. M.; Dugar, S.; Grazier, N.; Ngu, K.; Wu, S. C.; Yost, K. J.; Chen, B.-C.; Gougoutas, J. Z.; DiMarco, J. D.; Lan, S.-J.; Gavin, B. J.; Chen, A. Y.; Dorso, C. R.; Serafino, R.; Kirby, M.; Atwal, K. S. *J. Med. Chem.* **2001**, *44*, 3302.
7. Silveira, C. C.; Bernardi, C. R.; Braga, A. L.; Kaufman, T. S. *Tetrahedron Lett.* **2001**, *42*, 8947.
8. Kordik, C. P.; Luo, C.; Zanoni, B. C.; Dax, S. L.; McNally, J. J.; Lovenberg, T. W.; Wilson, S. J.; Reitz, A. B. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2283.
9. Redman, A. M.; Dumas, J.; Scott, W. J. *Org. Lett.* **2000**, *2*, 2061.
10. Burness, D. M. *J. Org. Chem.* **1956**, *21*, 102.
11. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
12. Pouysségur, J.; Sardet, C.; Franchi, A.; L'Allemain, G.; Paris, S. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 4833.
13. Laeokmann, D.; Rogister, F.; Dejardin, J.-V.; Prosperi-Meys, C.; Géczy, J.; Delarge, J.; Masereel, B. *Bioorg. Med. Chem.* **2002**, *10*, 1793.
14. Hove, M.; Van Emous, J. G.; Van Echteld, C. J. A. *Mol. Cell. Biochem.* **2003**, *250*, 47.
15. Miura, T.; Ogawa, T.; Suzuki, K.; Goto, M.; Shimamoto, K. *J. Am. Coll. Cardiol.* **1997**, *29*, 693.